Bivalve Bioaccumulation Correlation Analysis

Cannelton Industries Superfund Site

Tannery Bay, Sault Ste Marie, MI

Prepared for

National Oceanic and Atmospheric Administration

Coastal Protection and Restoration Division

77 West Jackson Boulevard SR 6J

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1.0 INTRODUCTION

The purpose of this document is to present the results of the 2004 bivalve bioaccumulation correlation study conducted in support of the long-term biomonitoring program for the Cannelton Industries, Inc. site, located along the St. Mary's River, Sault Ste. Marie, Michigan. The site biomonitoring program, a requirement of the Amended ROD (US EPA, 1996), was designed to monitor changes in the bioavailability of chromium, lead, cadmium, arsenic, and mercury in Tannery Bay sediments over time to verify whether the selected remedy for the site is effective at reducing concentrations of bioavailable trace elements in Tannery Bay.

Per the approved Operations & Management Plan, a minimum of three *in situ* biological monitoring events, using caged bivalve deployments, was to be conducted in order to collect sufficient data to evaluate bioaccumulation of site-related contaminants-of-concern into organisms. In 1997, during the study design phase, discussions were held with US EPA, NOAA, and Michigan Department of Natural Resource personnel to identify an appropriate species for the proposed caged bivalve study. The Asian clam, *Corbicula fluminea* (*Corbicula*), was identified as the best candidate because it is used extensively for biomonitoring and is readily available. While exotic to the region, the consensus at the time was that Corbicula was unlikely to spawn during the proposed deployment period (July to September) and, if juveniles were released, they could not survive the cold winter conditions. To date two bivalve monitoring events have been completed, a baseline event in 1997 and one post-construction-completion event in 2000 (NOAA and EVS, 1998; Applied Biomonitoring and HydroQual Incorporated, 2002). The 1997 and 2000 deployments used *Corbicula* collected from the Saline and Strawberry Rivers, Arkansas, respectively. A change in source clams in 2000 was necessitated by elevated concentrations of mercury in the Saline River *Corbicula* compared to the study site.

The second round of post-construction biological monitoring was scheduled for 2003; however, due to difficulties obtaining test organisms, low water levels, and potential implementation of a Tannery Bay dredging proposal under the Great Lakes Legacy Act, the event was postponed. By late 2002, *Corbicula* had expanded its range into northern Wisconsin lakes, which are within a degree latitude south of Sault Ste. Marie. This conflicted with information regarding the life history of *Corbicula* known at initiation of the monitoring program and raised concerns of introducing the species into the St. Mary's River. Therefore, a change in monitoring organism was necessitated to continue the program. The indigenous mussel, *Elliptio complanata* (*Elliptio*),

was identified as a replacement monitoring organism. To determine whether historical *Corbicula* monitoring data could be compared with future data collected using *Elliptio*, this contaminant bioaccumulation correlation study was conducted.

This correlation study was implemented to evaluate the relative net contaminant bioaccumulation between the *Corbicula* collected from the two sources and between the *Corbicula* and the *Elliptio* species. This study evaluated whether the resident mussel species is sufficiently similar in its bioaccumulation of contaminants from site sediments to allow the "normalization" of historical data collected using the Asian clam in previous sampling events to new data from future *Elliptio* deployments. In 2004, NOAA initiated the bioacummulation study of selected metals and arsenic by exposing the bivalves to contaminated sediment from Tannery Bay in laboratory mesocosms. The study design included four treatments consisting of one control and three treatments with contaminated sediment (Table 1). The bioaccumulation of chromium, the metal of primary concern at the Cannelton Site, during the 55-day study was statistically analyzed to provide equations that relate bioaccumulation between the different bivalve sources. This report summarizes the results of that study and provides the mathematical equations developed for chromium that can be used to relate monitoring data from both past and future deployments for a trend analysis.

2.0 METHODS

2.1 Exposure Methods

The bivalve exposures were conducted by staff at Arkansas State University (ASU) in flow-through mesocosms at the Mammoth Spring National Fish Hatchery in Mammoth Spring, AR from July 9 to September 2, 2004. Pictures of the experimental setup and procedures are presented in Appendix A. In addition to the 55-day bivalve exposure, six days were required to initiate the test and two days to conduct end-of-test measurements and sample preparation. The mesocosm treatment protocols replicated the organism-handling procedures of the 1997 and 2000 field deployments. The mussel and clam transplant methodology for this exposure was based generally on the methods described in the Guide for Conducting Field Bioassays with Marine, Estuarine, and Freshwater Bivalves (ASTM 2002), with the exception that exposures to field sediments were made in mesocosms rather than at the actual field site.

Parameters used to assess bivalve condition and responses to mesocosm exposure conditions included mortality, percent moisture, and changes in whole animal wet-weight (WAWW), as well as soft tissue and shell weight. WAWW was measured for bivalves prior to and following exposures. Length measurements were used only for initial sorting prior to deployment to ensure similar variance among replicates. Individual bivalve tissue and shell weights were recorded for dissected bivalves in preparation of composite samples for shipment.

2.1.1 Test Organisms

Corbicula were collected by Arkansas State University (ASU) researchers from the Strawberry River, located in the Ozark Highland Eco-region of north central Arkansas, on June 23, 2004**.** Clams in the size range of 1.5- to 2-cm were collected at a site from which clams were collected previously (2000 biomonitoring event) and were previously shown to be free of anthropogenic contamination, as well as free of disease and pest species, e.g., Zebra mussels. Approximately 1,500 clams were collected, placed in insulated, aerated coolers containing Strawberry River water and transported by automobile to the hatchery.

Corbicula were also collected from the runs and riffles in the North Fork Saline River of the Ouachitas region in Arkansas, on June 25, 2004. Approximately 1,500 clams in the 2- to 3-cm size range were also collected at a site from which clams were collected previously (1997 baseline event) and were shown to be free of disease and pest species. Following collection,

clams were placed in insulated, aerated coolers containing Saline River water and transported by automobile to the hatchery.

Elliptio were collected by divers on June 30, 2004, from Balsam Lake, Ontario, Canada, wrapped in brown paper towels and packed between wet burlap bags on top of ice in coolers, which were then shipped the same day by overnight courier to the hatchery (Appendix B). The mussels were received on July 1, 2004.

Immediately upon arrival at the hatchery, the mussels and clams were removed from the coolers, inspected for condition, and placed directly into the control mesocosm, which was maintained at 15-20°C and sufficiently oxygenated with an air stone to ensure bivalve survival and consistency with earlier deployment methods. Dead and stressed individuals, i.e., those that were gaping or failed to close their shells upon light physical stimulation, were discarded. The clams and mussels were allowed to acclimate and purge their guts for three days prior to sediment exposures in the control tanks and were allowed to feed only on particles associated with the ambient conditions of the water flowing to the mesocosms.

Care and handling of the bivalves throughout the sorting, distribution, and placement processes ensured test animals of high quality and without stress. During the sorting process, bivalves were held in aerated coolers and were never out of water for longer than 30 minutes to minimize handling stress. Following initial sorting, clams and mussels were placed back into the coolers containing aerated hatchery water. The undistributed bivalves were held in coolers throughout the measurement and distribution process. Only live animals, which were fully closed, or those that closed immediately upon light physical stimulation, were used in exposures.

Prior to test organism selection, calipers were used to establish appropriate bivalve size ranges and individual organisms were selected from this cohort. Mussels in the 4.5- to 6.5-cm size range were selected. Clams ranging from 1.5- to 3.0-cm length were selected from the Saline and Strawberry River source groups and sorted to provide sizes similar to the size classes deployed in Tannery Bay from the Saline River in 1997 and those deployed from the Strawberry River in 2000.

2.1.2 Sediment Collection and Mesocosm Preparation

Sediment for the bioaccumulation correlation study was collected on June 8, 2005, in coordination with a site inspection that was conducted in support of the site 5-Year Review. Staff from NOAA, HydroQual, Inc., and Conestogo-Rovers & Associates (CRA) physically inspected Tannery Bay sediment conditions, water depths, and other factors to plan for the biological monitoring program. Water depths throughout the bay appeared consistently shallower compared with depths from the 1997 baseline biological monitoring event. The shallower depths were most noticeable along the eastern shore of the bay, where sedimentation had filled a previous six- to eight-foot-deep channel to approximately two feet deep or shallower. Due to low lake levels, the water depths along the western and southwestern wetland shore varied from zero to approximately eight inches. Water depth fluctuations in this corner of the bay are common due to variations in lake levels, wind-driven waves, and waves from commercial ship traffic.

Sediment collection locations were chosen based on historical chromium concentrations as indicated by previous monitoring activities (Table 2). Sediments were collected near historical Stations 2, 3, 5, 7, 9, and 10 (NOAA and EVS, 1998; Applied Biomonitoring and HydroQual Incorporated, 2002; Figure 1). Target collection sites were located by CRA using a Leica sub-meter global positioning system receiver. Sediments from each target location were collected by shovel and placed in a 3 mil plastic bag lining a 24-liter cooler. Once each cooler was filled, the plastic bag was tied or sealed with tie-wraps. Coolers were labeled with H-, M-, or L- to designate the expected relative chromium concentration, based on the previous sampling, followed by the site location, and cooler number collected at that location.

Sediment collection began at 1020 hours at Station S3 and was completed at location S2 at 1435 hours. Coolers were transported to a staging site in east Sault Ste. Marie for external labeling and shipping preparation. Coolers were shipped June 9 via FedEx to the USFWS National Fish Hatchery in Mammoth Springs, AR. The sediments at the time of sampling ranged from 16 to 20 ^{o}C. Hard blue ice packs were placed on top of the plastic bags lining the coolers to control temperature during shipment. Sediments were received on June 11 at the hatchery.

Initial sediment chromium concentrations for each of 25 coolers were measured on a Varian AA Spectrometer (level of detection = 0.0017 mg/L) following sediment digestion procedures as described by Hannigan and Sholkovitz (2001). Based on these preliminary results, the coolers

were grouped into three targeted treatments according to relative chromium concentration (low, medium, and high; Table 2). All coolers from a specific treatment group were emptied into a large fiberglass container and the sediment was blended by hand with polycarbonate canoe paddles. The blended sediment from each treatment was distributed into a separate fiberglass mesocosm tank measuring 0.53 x 0.61 x 3.05 m. Sediments were mixed again in the tanks, allowed to settle, and leveled to a depth of 7.5 cm.

Flowing water for the mesocosms came from the Spring River and a pond at the hatchery. The water from those sources was supplied to a head tank and distributed into the four individual mesocosms to a depth of 30 cm overlying the sediment at a renewal rate of 7.5 L/ minute. Flow was adjusted between the river and pond waters to maintain a target water temperature of 18.0°C in the mixing reservoir. Water in each mesocosm was maintained between 18.0 and 18.5°C to mimic Tannery Bay deployment conditions. Algae in the pond water served as a food source for the bivalves deployed.

The mesh tubes containing mussels and clams were placed in the mesocosms. At least 3 cm was maintained between each tube and they were laid carefully on top of the sediment to avoid being stretched taut or have a large amount of slack. Pictures of the experimental setup and bivalve handling are presented in Appendix A.

Additional sediment samples were collected from each treatment mesocosm after sediment settling but prior to bivalve deployment and again just after bivalve retrieval. These samples were shipped overnight to the Battelle Marine Sciences Laboratory, Sequim, WA (Battelle). The samples collected at the start of the exposure were frozen and held by the laboratory until all samples were received at the end of the testing.

2.1.3 Bivalve Distribution

To ensure selection of mussels of a similar age group, only individuals from 7.5 – 25 g WAWW were used for this study. Clams retrieved from the Strawberry and Saline rivers ranged from 1.2 – 5.5 g and 3.5 – 10.7 g WAWW, respectively. Bivalve size varied from the proposed ranges due to the availability of test organisms.

The bivalves were blotted dry individually prior to being placed on a Shimadzu UX420H electronic scale. Individual bivalve WAWWs were measured and recorded electronically into an Excel spreadsheet to the nearest 0.001 g and then the bivalve was placed into mesh tubing.

To provide the start-of-test (T_0) measurements and tissue chemistry, subsets of clams and mussels representing a T_0 treatment were sequentially weighed and distributed to labeled, compartmentalized trays to facilitate processing rather than being distributed into mesh tubes. Measurement and distribution of all bivalves used for T_0 occurred simultaneously with bivalve deployment in mesocosms. Due to an oversight, these individuals included only one replicate of 20 mussels and one replicate of 50 clams from each of the two clam sources, instead of the intended 3 replicates from each bivalve group. The consequences of this omission are discussed in Section 3.5, below.

After weighing, the other test bivalves were placed directly into mesh tubes that were marked with treatment number and tube replicate. Nylon cable ties separated individuals and prevented bivalves from shifting position in the tubes. Cable ties were bound loosely enough to allow free movement while maintaining equal bivalve separation and ensure equal exposure to bioavailable metals and arsenic. The mesh tubing allowed good water circulation and exposure to environmental conditions. A total of 3 replicates (50+50+20 each) from each of the three bivalve groups comprised the nine mesh tubes prepared for each treatment resulting in a total of 360 animals per treatment (Table 1).

One-way ANOVAs were used to verify that the T_0 bivalves were statistically the same sizes as the bivalves to be exposed. Following statistical analyses, the T_0 mussels and clams, viz., one replicate of 20 mussels and one replicate of 50 clams for each of the two source groups, were prepared, measured, and submitted for chemical analyses as described below. While maintaining proper sequence, tissues were removed from valves of each mussel and clam by replicate, and both the individual tissues and shells weighed separately as described in detail below. The T_0 tissue weights from each of the single replicate per bivalve source group were averaged and these means were used to estimate the soft tissue weights for all deployed individuals in each of the three source groups. Tissues from these individuals were composited by replicate and source group in pre-cleaned glass jars, frozen, and sent to Battelle Marine Sciences Laboratory, Sequim, WA, by overnight courier. The frozen T_0 tissue composites were held by the laboratory until all of the samples were received at the end of testing.

2.1.4 Water Quality Parameters

Temperature, pH, dissolved oxygen (DO), and conductivity were measured in source, head tank, and overlying mesocosm waters on days 1, 6, 15, 22, 34, 42 and 55. Water was collected

and returned to ASU Ecotoxicology Research Facility for measures of alkalinity and hardness on days 1, 6, 15, and 55. All water quality test methods followed American Public Health Association (1998) guidelines utilizing a YSI Model 85 multi-meter, and an Accumet AR 25 dual channel pH meter. The water quality data are summarized in Appendix C.

2.1.5 Bivalve Retrieval, Measurements, and Sample Preparation

Following 55 days of exposure to treatment sediments, bivalves were retrieved from the mesocosms and allowed to purge overnight in separate clean systems prior to processing. Retrieval was initiated the morning of September 2, 2004. At that time, the bivalves from each mesocosm exposure were place in 47-L Colemen® coolers (0.38 x 0.57 x 0.43 m) containing water from the respective mesocosms. Continual aeration was supplied to each cooler via a Dual Air Pump®. Coolers were then transported by automobile to ASU Ecotoxicology Research Facility where they were allowed to gut-purge overnight. This period replicated the procedure used in the field deployments.

Final bivalve weighing, dissection, and tissue retrieval proceeded the following day (September 3, 2004). Two teams equipped with electronic balances with laptop connections, aluminum trays for bivalve sorting, and dissection tools were assembled. One team dissected and weighed the bivalves from the control exposure tank and then from the medium concentration tank. The second team dissected and weighed the bivalves from the low concentration tank, then from the high concentration tank. The replicate and order were clearly marked on the sleeves, and care was taken to retrieve bivalves from the sleeves in the precise order as placement into the sleeves during test setup.

All instruments used in tissue extraction were composed of corrosion resistant stainless steel, anodized aluminum, or borosilicate glass. Following the procedures in the field plan, all instruments and surfaces were thoroughly cleaned at the start of the shucking process and after all mussels from a given treatment were processed. Cutting surfaces were covered with clean cloth towels. Gloves worn during the shucking process were powder free. Technicians cleansed hands thoroughly with a phosphate-free detergent or replaced gloves and towels prior to shucking mussels from a new treatment. Exposure of bivalve tissue to hands, towels, and any surface other than the interior of the specimen's original shell was minimized at all times.

Tissue processing included all bivalves from one treatment as a unit. Mussels or clams that did not completely close their shells upon light physical stimulation were considered dead and

were discarded without measurements. During tissue collection, the mussels and clams were maintained in sequence; individual tissue weights were recorded and paired with WAWW and shell weights. Tissue extraction was accomplished by placing an individual bivalve on a cutting surface and severing the posterior and anterior adductor muscles by sliding a stainless steel knife blade between the valves. The tip of the knife blade was then used to separate tissue from shell; as much of the adductor muscle as possible was severed from the points of attachment and was scraped from the shell. Excess liquid was drained by holding tissues against the shell with the knife blade, tipping the shell slightly, and dabbing onto a clean towel.

Detached tissues were kept in their original shell following separation until tissue weights were made. The shucked visceral mass, held in the shell, was placed on an aluminum tray lined with paper towels marked with the appropriate number. Sufficient space was allowed between individuals to prevent contamination of soft tissue from adjacent individuals.

This process was repeated until viscera from all bivalves in a given treatment were retrieved and compiled into pre-cleaned glass jars. All soft tissues from a source group were compiled for each treatment and individual tissue weights obtained by taring the weighing pan prior to the addition of each tissue mass. As tissues were added, the individual weights were recorded electronically to a spreadsheet as previously described.

Bivalves were then weighed to the nearest 0.001 g sequentially following tissue extraction. Each bivalve was blotted dry just prior to placement on a balance and recorded electronically into a spreadsheet. All sampling equipment was thoroughly cleaned before proceeding to next sample.

Following weight recordings of individuals from each replicate, the sample jar was tightly capped, a pre–labeled sticker affixed, tamper-proof tape applied across the lid, and the sample placed in a -20°C freezer until shipped. Sample jars were wrapped with bubble-wrap and packed into a cooler. A sufficient amount of hard blue ice was packed around the sample jars to keep them frozen during shipment. The coolers were delivered by FedEX via overnight delivery to Battelle.

2.1.6 Quality Assurance/Quality Control Procedures

Quality assurance (QA) and quality control (QC) procedures were employed during the measurement of bivalves and chemical analysis. Precision was assured during weight measures

as follows. For at least every 60 WAWWs recorded, five individuals were re-measured and fell within 10% variance. These check data were not retained. Accuracy of the electronic balances was checked periodically during use with a standard weight. All checks also fell within 10% variance.

All WAWW, shell weight, and visceral mass measurements were recorded electronically into an Excel spread sheet. The accurate numerical transfer from the balance to the spreadsheet was checked each time visually by the weighing technician.

2.1.6.1 Decontamination Procedures

All sample equipment, cutting surfaces and stainless steel shucking knives were decontaminated between treatments by washing in the sequence of phosphate-free detergent, tap water, 10% nitric acid and distilled water. Latex gloves were changed between each treatment.

2.1.6.2. Documentation

All field collection and shipping activities were documented in lab notebooks, data sheets, and electronic files. Documentation included records of the field collections, exposure set up and monitoring data, samples collected for analysis, sample labels and tracking forms, COC forms, and photographic documentation.

Chain-of-custody (COC) provided traceable documentation from collection through laboratory analysis according to the work plan. Completed COC forms were placed in a plastic envelope and taped to the inside lid of the cooler containing the listed samples during overnight delivery via FedEX. The lid of the cooler was sealed with fiber tape on two sides and a "Glass–Handle with Care" label was attached to the top of the cooler and sealed with a custody seal. Copies of COC forms are included as Appendix D.

2.2 Chemical Analyses Methods

The sediment samples were thawed at the laboratory, homogenized thoroughly, and analyzed for arsenic, cadmium, chromium, lead, and moisture content. The bivalve composites were also thawed, thoroughly homogenized, and analyzed for arsenic, cadmium, chromium, lead, lipids, and moisture content. Methods that were used for the tissue and sediment analyses, the targeted detection limits, and other data quality objective information are presented in Table 3.

2.3 Statistical Analyses Methods

2.3.1 Bivalve Growth

Change in mean whole-body mass by combination of bivalve type and sediment type was used to assess the health of bivalves. Box plots were used to summarize individual changes in bivalve whole-body mass. As whole-body mass measurements are non-destructive they were made on individual bivalves before and after the exposures. In comparison, the initial visceral and shell mass T_0 averages were estimated from one T_0 replicate, as the other two replicates were not collected. Thus, changes in individual whole-body mass were measured with greater accuracy and precision than changes estimated in visceral and shell mass.

For each bivalve type (Balsam Lake *Elliptio*, Saline River *Corbicula*, and Strawberry River *Corbicula*), a one-way ANOVA (Neter 1996) was used to test whether changes in whole-body mass at low, medium, and high sediment chromium concentrations differed from change in mass at the control level.

Post-treatment mean visceral and mean shell mass were compared to corresponding baseline means (T_0 bivalves) by combination of bivalve type and sediment type.

2.3.2 Accumulation of Metals and Arsenic

Uptake relationships among the bivalve groups were statistically tested for Cr, the primary contaminant of concern. These tests were preformed using the end-of-test data. No statistical tests were made between the concentrations of trace elements at the beginning and end of the exposures because multiple T_0 replicates were not obtained.

Each mesh tube replicate was treated as the primary experimental unit (n=36). There were two bioaccumulation variables of interest: change in Cr concentration (mg/g) and change in Cr mass (mg). For each bioaccumulation variable, linear regression (Neter 1996) was used to estimate a conversion equation for *Elliptio* and *Corbicula* Cr bioaccumulation. The relationship was of the form:

$$\ln(\Delta Cr_{Corbicula}) = \beta_0 + \beta_1 \ln(\Delta Cr_{Elliptio}) + \beta_2 (Source) + \beta_3 \ln(\Delta Cr_{Elliptio}) \times (Source)$$

where, ΔCr represents change in *Cr* concentration or mass and *Source* is defined to be 1 for *Corbicula* from the Strawberry River and 0 for Saline River *Corbicula*. This term allows the

model to describe relationships that may differ among *Corbicula* populations. When the relationship differs by source (i.e., rejection of null hypothesis that $\beta_2 = \beta_3 = 0.0$), the model provides a separate conversion equation for each source of *Corbicula*. When the relationship is similar by source, the model simplifies to one conversion equation for both *Corbicula* sources.

All statistical analyses were performed using the open source statistics language R version 2.0.1 (R Development Core Team 2004).

3.0 RESULTS

3.1 Bivalve Growth

Growth data are presented in Appendix E. There was little change in mean whole-body mass for combinations of bivalve type and sediment type (Table 4). The largest average gain was 2.5% for Strawberry River *Corbicula* in the high Cr concentration tank. The largest mean loss was -2.1% for *Elliptio* at the control Cr level. It appears that the algae in the pond water were insufficient to sustain bivalve weight. On an individual clam basis, percent change in whole-body mass tended to be small (Figures 2 to 4), but high variance was observed for Strawberry River *Corbicula* in the control and high Cr concentration tanks (Figure 4). This elevated variance was restricted to high values in Replicate 2 of the control tank (Figure 5 panel B) and Replicate 3 of the high Cr concentration tank (Figure 5 panel L). The high variance in these replicates is most likely explained by measurement discrepancies that could be related to a loss of integrity in clam order, and not by changes in clam weight. Mean changes in whole-body mass were also calculated excluding the high variance replicates (Table 4). *Elliptio* whole-body mass declined in all of the sediment concentrations: Compared to the mean loss in *Elliptio* mass in the control tank (-0.294 g), mean loss was smaller in the low (-0.243 g), medium (-0.224 g), and high (-0.095 g; $p=0.002$) Cr concentration tanks. Whole-body mass in Saline River *Corbicula* increased in the control tank (0.001 g) and decreased ($p<0.009$) in the low (-0.022 g), medium (-0.022 g), and high (-0.011 g) Cr concentration tanks. Whole-body mass of Strawberry River *Corbicula* increased similarly ($p>0.17$) in all 4 tanks: control (0.037 g), low (0.027 g), medium (0.054 g), high (0.068 g). Calculations made by excluding the two high-variance Strawberry River replicates, indicate that increases in mean whole-body mass in the low (0.026g) and control (0.017g) tanks were similar, but increases in the medium (0.054g) and high (0.066g) tanks differed ($p\leq0.001$) from control. These data show that growth in all exposures was similar to the control growth.

Changes in mean visceral mass (Table 5) were more variable than changes in whole-body mass, ranging from a gain of 21.3% for *Elliptio* in the control treatment to a loss of 17% for Saline River *Corbicula* in the control treatment. Similar variability was observed for change in mean shell mass (Table 6) ranging from a gain of 22.4% for *Elliptio* in the control treatment to a loss of 22.8% for Saline River *Corbicula* in the control treatment. The combined change in mean visceral and shell mass measurements was inconsistent with corresponding change in whole-body mass measurements. The differences were most notable for *Elliptio* in which changes in mean visceral and shell mass were positive for all sediment concentrations (Tables 5 and 6), but changes in

mean whole-body mass were negative for all sediment concentrations (Tables 4). For Strawberry River *Corbicula*, the combined change in mean visceral and shell mass greatly exceeded mean change in whole-body mass for all sediment concentrations. By contrast, for Saline River *Corbicula*, the combined change in mean visceral and shell mass was much smaller than mean change in whole-body mass for all sediment concentrations. Although care was taken to retrieve and measure bivalves in the original sequence, it appears that the integrity of the bivalve sequence was not maintained in all replicates.

3.2 Accumulation of Metals and Arsenic

The detailed result of the chemical analyses, including associated QA data, are presented in Appendix F, and summarized in Figure 6. All data quality objectives for these analyses were met, with the exception that only one replicate tissue composite was generated for each T_0 bivalve group.

Overall the data showed (based on non-statistically based observations):

- That the concentration ranges of the metals and arsenic measured in the sediments were representative of the ranges of concentrations observed in Tannery Bay.
- That the concentrations of arsenic and cadmium in the tissues of all three bivalve groups did not change substantially during the 55-day exposure, consistent with the limited range of concentrations exhibited by the sediments.
- That the concentrations of lead increased in the bivalves to a limited extent as a result of exposure to the sediment with the highest lead concentration, but not in the lower exposures.
- That the concentrations of chromium increased in all bivalves proportional to the concentrations in the sediments to which they were exposed.
- That, when substantial bioaccumulation occurred, viz., of chromium and lead, the two *Corbicula* groups had higher bioaccumulation on both a concentration and content basis than *Elliptio*, and the smaller clams from the Strawberry River had higher bioaccumulation on both a concentration and content basis than did the clams from the Saline River.

Based on these observations, the bioaccumulation of the primary contaminant of concern, chromium, was tested statistically for a relationship between the three different groups of bivalves.

3.3 Conversion Equations for Change in Cr Concentration (mg/kg)

On a concentration basis, the relationship between *Corbicula* and *Elliptio* Cr bioaccumulation was reasonably described (adjusted R^2=0.93) by the log-log relationship:

$$\begin{aligned}\ln(\Delta Cr_{Corbicula}) = &-0.700 + 1.59 \times \ln(\Delta Cr_{Elliptio}) \\ &+ 0.507 \times (Source) \\ &+ 0.0 \times \ln(\Delta Cr_{Elliptio}) \times (Source).\end{aligned}$$

The regression coefficient estimates and standard errors are summarized in Table 7 and the fitted functions are plotted in Figure 7.

In the original concentration scale (as opposed to log scale), the equation for converting Strawberry River *Corbicula* change in concentration ($\Delta Cr_{Corbicula}$) to *Elliptio* change in concentration ($\Delta Cr_{Elliptio}$) is:

$$\Delta Cr_{Elliptio} = \exp\left[\frac{\log(\Delta Cr_{Corbicula}) + 0.193}{1.590}\right].$$

The equation for converting Saline River *Corbicula* change in concentration ($\Delta Cr_{Corbicula}$) to *Elliptio* change in concentration ($\Delta Cr_{Elliptio}$) is:

$$\Delta Cr_{Elliptio} = \exp\left[\frac{\log(\Delta Cr_{Corbicula}) + 0.700}{1.590}\right].$$

3.4 Conversion Equations for Change in Cr Mass (mg)

The log-log relationship between change in Cr mass in *Corbicula* and *Elliptio* was similar (p=0.125) for both Strawberry and Saline river source populations. The relationship between *Corbicula* and *Elliptio* change in Cr mass was best described (adjusted R^2=0.87) by the log-log relationship (Table 8):

$$\ln(\Delta Cr_{Corbicula}) = -0.969 + 1.411 \times \ln(\Delta Cr_{Elliptio}).$$

In the original mass scale (as opposed to log scale), change in mass for *Corbicula* (mg $Cr_{Corbicula}$) can be converted to *Elliptio* scale change in mass (mg $Cr_{Elliptio}$) using the equation, plotted in Figure 8:

$$\Delta Cr_{Elliptio} = \exp\left[\frac{\log(\Delta Cr_{Corbicula}) + 0.969}{1.411}\right].$$

3.5 Effect of Single T_0 Replicate

The absence of multiple T_0 replicates did not affect the derivation of the Cr bioaccumulation relationships. Failure to replicate the T_0 group did not adversely affect estimates of the regression equations because the regression relies on an average of T_0 and is therefore, unbiased regardless of the number of replicates. The size distribution of the individual organisms within the single T_0 replicate is based on a large sample number, specifically, 20 or 50 individuals, providing a representative estimate of the test organisms. The absence of multiple T_0 replicates precludes a more precise estimate of initial variability, which results in a tendency to underestimate standard errors of the regression coefficients (Tables 7 and 8). This effect is likely to be small but the potential range of error cannot be quantified.

4.0 CONCLUSION

The question posed by this study was whether data from past monitoring events can be related to data from future events using a different species, viz., *Elliptio*. This study demonstrated that the relative bioaccumulation of chromium concentration and content was comparable between *Corbicula* and *Elliptio* and was directly related to the concentrations in the exposure sediment.

A quantitative bioaccumulation relationship between the bivalve groups was established statistically. Therefore, future trend monitoring based on *Elliptio* can be reliably related to past *Corbicula* data.

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TABLES

Table 1. Study Design for Mammoth Spring Hatchery Mesocosms

Treatment Sediments Shipped, Mussels Collected and Shipped, Clams Collected	Provided By NOAA and ASU, Acclimation and Settling at Hatchery
Surface Water Samples from mesocosms	Collected seven times (approximately weekly), on days 1, 6, 10, 22, 34, 42, and 55
Sediment Samples from mesocosms	Collected three times: 1) at mixing stage to verify concentrations were in desired ranges, 2) after sediment settling but prior to bivalve deployment, 3) just following bivalve retrieval
Size range of mussels at start of test	Approximately 4.5 to 6.5-cm shell length; 2.0 to 4.0-g wet tissue-weight (7.5-25 g WAWW)
Size range of clams at start of test	Approximately 1.5-3-cm shell length and 0.25 – 1.00-g wet tissue-weight (1.2 – 5.5-g WAWW - Strawberry and 3.5 – 10.7-g WAWW - Saline)
Number of mussels per treatment	60 (3 tubes with 20 mussels/ tube)
Number of clams per treatment	300 (3 tubes with 50 clams/ tube) for each of 2 source/size groups
Number of replicates (sleeves) per mesocosm	9 (3 with mussels and 3 with each of the 2 clam groups)
Number used for T_0 tissue chemistry	3 reps of 20 mussels and 3 reps of 50 clams for each of the 2 source groups (only one rep from each bivalve group was collected)
Total number of mussels used	260 (20 + 4 x 60)
Total number of clams used	1250 (50 + 4 x 150 for each of the 2 source groups)
Exposure configuration	4 mesocosms: one control with no sediment and 3 with targeted order-of-magnitude differences in metal concentrations
Exposure period	55 days
Survival and growth	Survival, changes in weight (WAWW, tissue weight, and shell weight) were measured

Table 2. Sediment Collection and Distribution Data

Cooler No.	Cooler ID	Station Loc	Historical Cr Range ppm	Cr Concentrations in Overlying Water	Mesocosm Mix
1	H3.1+E	12' E of S3	~20,000	123	High
2	H3.2+E	12' E of S3	~20,000	133	High
3	H5-00.1	S5-00	12,000- 30,000	73.9	Medium
4	H5-00.2	S5-00	12,000- 30,000	127	Medium
5	H5-00.3	S5-00	12,000- 30,000	77.9	Medium
6	H5-00.4	S5-00	12,000- 30,000	121	Medium
7	H5-00.5	S5-00	12,000- 30,000	153	High
8	H5-00.6	S5-00	12,000- 30,000	164	High
9	H5-00.7	S5-00	12,000- 30,000	486	High
10	H4.5.1	~40mSE of S4 and 25m NNW of S5-00	11,000-18,000 and 12,000-30,000	335	High
11	H4.5.2	~40mSE of S4 and 25m NNW of S5-00	11,000-18,000 and 12,000-30,000	236	High
12	H4.5.3	~40mSE of S4 and 25m NNW of S5-00	11,000-18,000 and 12,000-30,000	813	High
13	M07.1	S7	2700 - 4500	128	Medium
14	M07.2	S7	2700 - 4500	83.1	Medium
15	M09.1	S9	950 - 990	43.2	Medium
16	M09.2	S9	950 - 990	44.2	Medium
17	M09.3	S9	950 - 990	32.4	Medium
18	M09.4	S9	950 - 990	43.8	Low
19	M10.1	S10	250 - 1200	12.9	Low
20	M10.2	S10	250 - 1200	24.3	Low
21	L02.1	S2	<13 - 17*	0	Low
22	L02.2	S2	<13 - 17*	0	Low
23	L02.3	S2	<13 - 17*	0	Low
24	L02.4	S2	<13 - 17*	0	Low

* Non-detect at 13 ppm.

Table 3. QA/QC Data Quality Objectives

Analyte	Reference Method	Method Detection Limit	Method Blank	Range of Recovery	SRM Accuracy	Relative Precision
Arsenic	GFAA (EPA 200.9)	0.2 mg/kg	≤5xMDL	75-125%	≤20%	≤20%
Cadmium	ICP/OES (EPA 200.7)	0.01 mg/kg	≤5xMDL	75-125%	≤20%	≤20%
Chromium	ICP/OES (EPA 200.7)	0.1 mg/kg	≤5xMDL	75-125%	≤20%	≤20%
Lead	ICP/OES (EPA 200.7)	0.01 mg/kg	≤5xMDL	75-125%	≤20%	≤20%

Table 4. Change in Mean Whole-Body Wet Weight (WAWW) by Bivalve Type and Sediment Type

Bivalve Type	Sediment Type	Mean Whole-Body Mass				ANOVA p
		Initial (g)	Final (g)	Difference (g)	% Change	
Elliptio	Control	13.87	13.58	-0.29	-2.1	<0.001
	Low	13.23	12.98	-0.24	-1.8	0.425
	Medium	13.28	13.06	-0.22	-1.7	0.274
	High	13.09	13.00	-0.09	-0.7	0.002
Corbicula (Saline River)	Control	5.74	5.74	0.00	0.0	0.653
	Low	6.71	6.69	-0.02	-0.3	<0.001
	Medium	6.54	6.52	-0.02	-0.3	<0.001
	High	6.32	6.31	-0.01	-0.2	0.009
Corbicula (Strawberry River)	Control	2.75	2.79	0.04	1.4	0.020
	Low	2.74	2.77	0.03	1.0	0.644
	Medium	2.72	2.78	0.05	2.0	0.455
	High	2.77	2.84	0.07	2.5	0.171
Corbicula (Strawberry River) less control replicate 2 and high replicate 3	Control	2.88	2.89	0.02	0.6	0.004
	Low	2.74	2.77	0.03	1.0	0.218
	Medium	2.72	2.78	0.05	2.0	<0.001
	High	2.80	2.87	0.07	2.4	<0.001

Table 5. Change in Mean Visceral Bivalve Mass by Bivalve Type and Sediment Type

Bivalve Type	Sediment Type	Mean Visceral Mass			
		Initial (g)	Final (g)	Difference (g)	% Change
Elliptio	Control	2.73	3.31	0.58	21.3
Elliptio	Low	2.73	3.06	0.34	12.3
Elliptio	Medium	2.73	3.02	0.29	10.8
Elliptio	High	2.73	3.10	0.37	13.6
Corbicula (Strawberry River)	Control	0.37	0.40	0.03	8.3
Corbicula (Strawberry River)	Low	0.37	0.39	0.02	6.4
Corbicula (Strawberry River)	Medium	0.37	0.39	0.02	4.9
Corbicula (Strawberry River)	High	0.37	0.42	0.05	13.2
Corbicula (Saline River)	Control	1.06	0.88	-0.18	-17.0
Corbicula (Saline River)	Low	1.06	1.00	-0.06	-5.7
Corbicula (Saline River)	Medium	1.06	0.99	-0.07	-6.9
Corbicula (Saline River)	High	1.06	1.01	-0.06	-5.3

Table 6. Change in Mean Shell Bivalve Mass by Bivalve Type and Sediment Type

Bivalve Type	Sediment Type	Mean Shell Mass			
		Initial (g)	Final (g)	Difference (g)	% Change
Elliptio	Control	5.30	6.49	1.19	22.4
Elliptio	Low	5.30	6.08	0.78	14.7
Elliptio	Medium	5.30	5.95	0.65	12.3
Elliptio	High	5.30	6.16	0.86	16.2
Corbicula (Strawberry River)	Control	1.80	1.89	0.09	4.8
Corbicula (Strawberry River)	Low	1.80	1.89	0.08	4.6
Corbicula (Strawberry River)	Medium	1.80	1.89	0.08	4.5
Corbicula (Strawberry River)	High	1.80	1.93	0.13	7.0
Corbicula (Saline River)	Control	4.59	3.55	-1.05	-22.8
Corbicula (Saline River)	Low	4.59	4.09	-0.50	-11.0
Corbicula (Saline River)	Medium	4.59	4.00	-0.60	-13.0
Corbicula (Saline River)	High	4.59	3.88	-0.72	-15.6

Table 7. Regression to Estimate Conversion Equation for Change in Cr Concentration

Response	Predictor	Estimate	Std. Error	t	Pr(>|t|)
log *Corbicula* Cr (µg/g, dw)	Intercept	-0.700	0.214	-3.277	0.004
	log *Elliptio* Cr (µg/g, dw)	1.590	0.092	17.202	<0.001
	Strawberry River Indicator	0.507	0.239	2.116	0.046

t = Student's t statistic from a 2-sided significance test of whether a regression coefficient equals zero (null hypothesis)
Pr(>|t|) = Probability of observing a Student's t value greater in magnitude than the observed t statistic when the null hypothesis is true, i.e., p value. Small p values are evidence against the null hypothesis

Table 8. Regression to Estimate Conversion Equation for Change In Cr Mass

Response	Predictor	Estimate	Std. Error	t	Pr(>|t|)
log *Corbicula* Cr (µg, dw)	Intercept	-0.969	0.155	-6.240	<0.001
	log *Elliptio* Cr (µg, dw)	1.411	0.114	12.340	<0.001

t = Student's t statistic from a 2-sided significance test of whether a regression coefficient equals zero (null hypothesis)
Pr(>|t|) = Probability of observing a Student's t value greater in magnitude than the observed t statistic when the null hypothesis is true, i.e., p value. Small p values are evidence against the null hypothesis

FIGURES

Figure 1. Sediment collection locations.

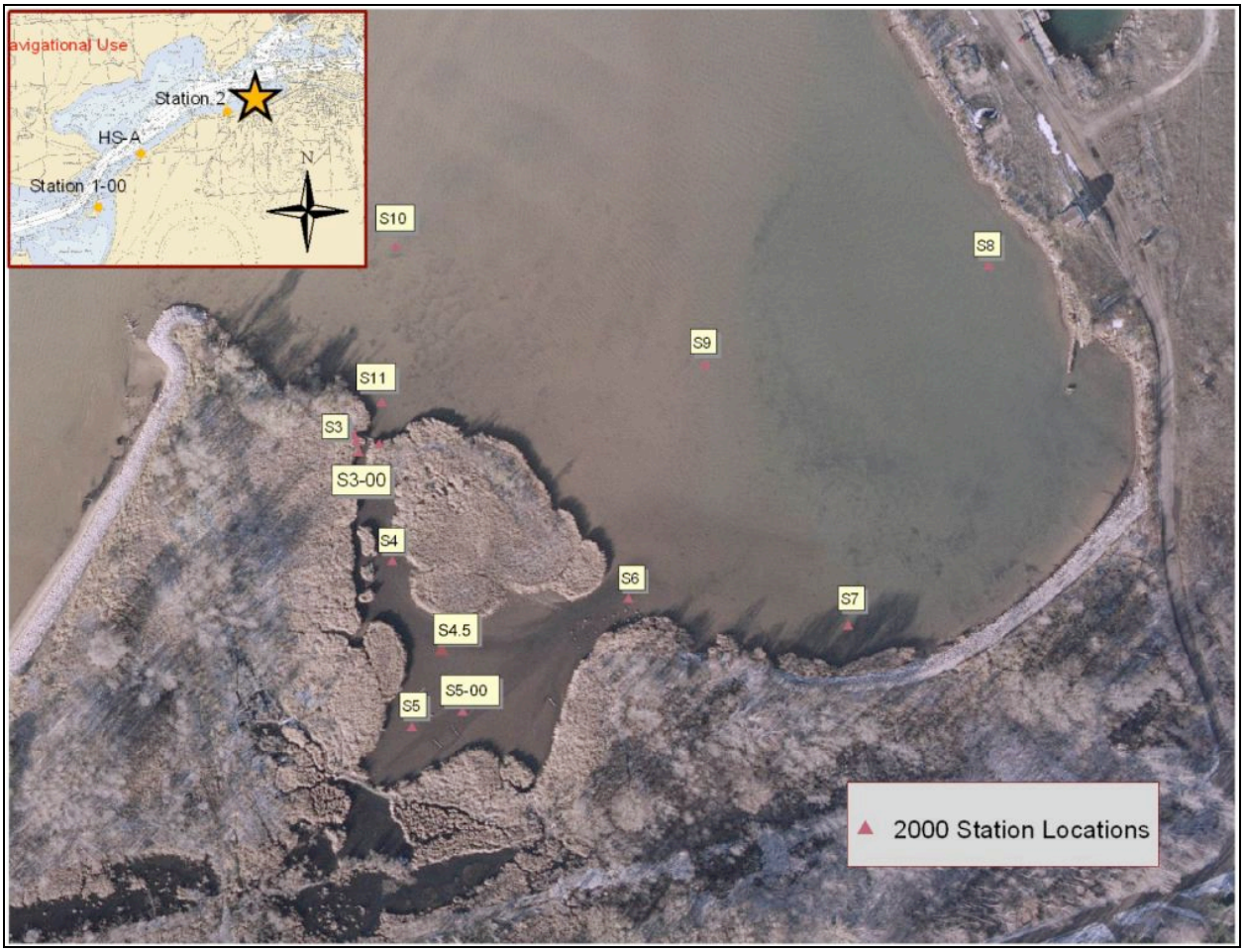


Figure 2. *Elliptio* change in whole-body wet weight.

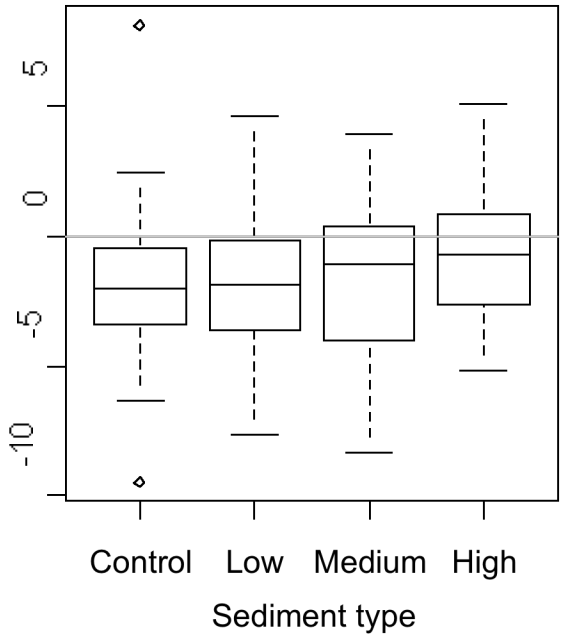


Figure 3. Saline River *Corbicula* change in whole-body wet weight.

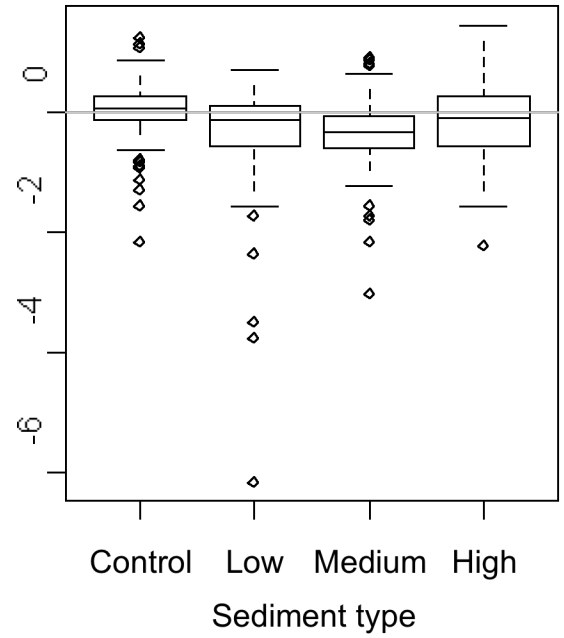


Figure 4. Strawberry River *Corbicula* change in whole-body wet weight.

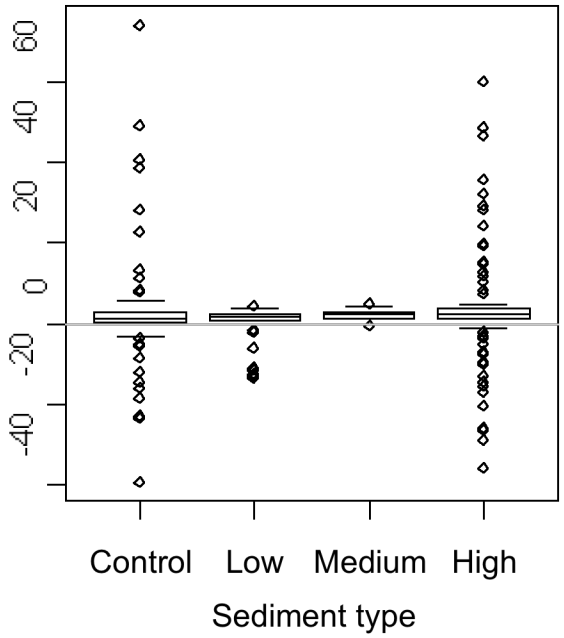


Figure 5. Strawberry River *Corbicula* T_0 whole-body wet weight (pre mass) versus end of test wet weight (post mass) by sediment type and replicate.

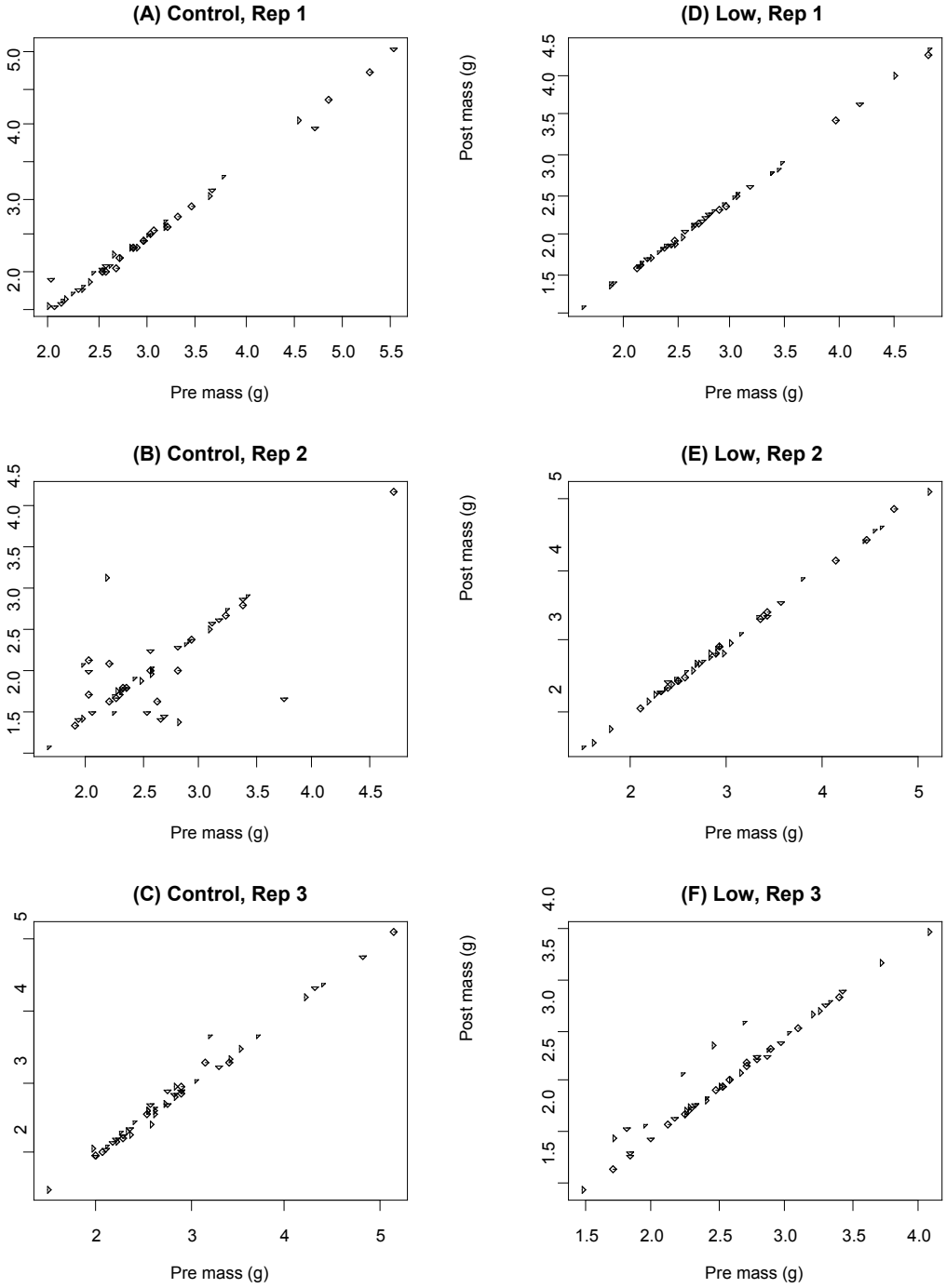


Figure 5 (continued). Strawberry River *Corbicula* T_0 whole-body wet weight (pre mass) versus end of test wet weight (post mass) by sediment type and replicate.

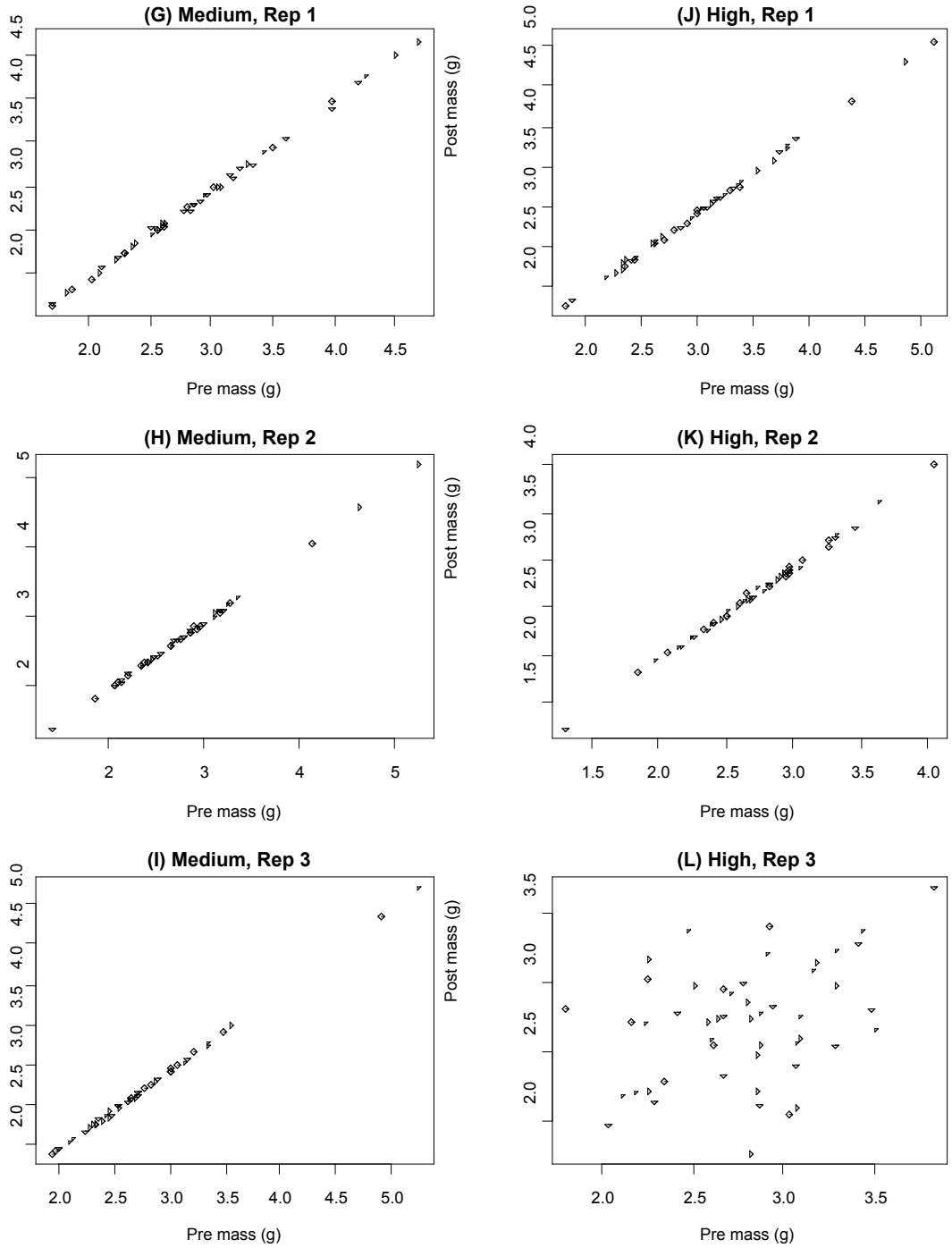
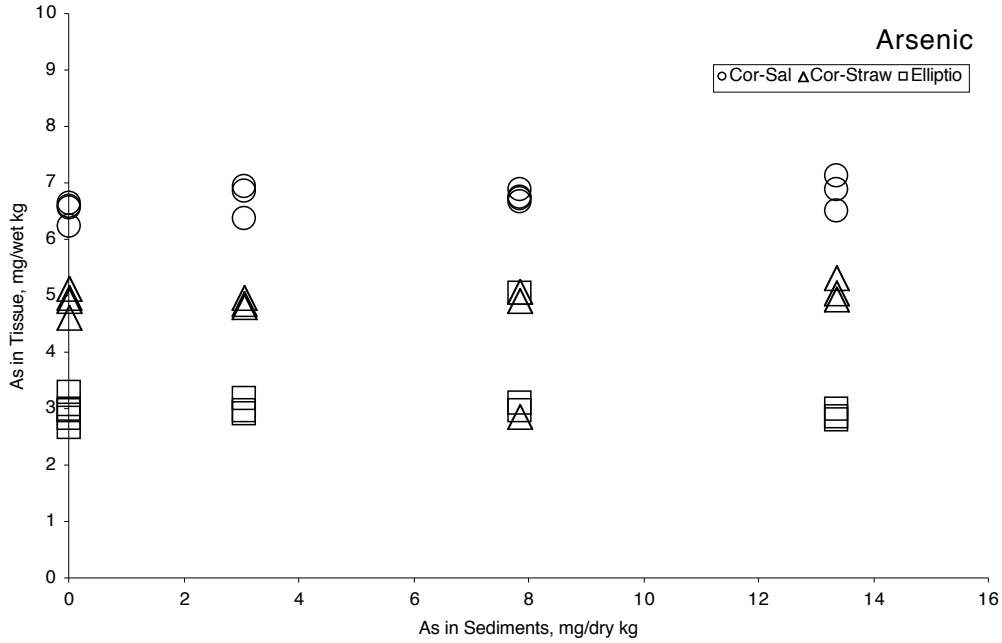


Figure 6. Comparison among the concentrations of the metals measured in the tissues of the bivalves to the concentrations in the sediments to which they were exposed. The sediment concentrations are the average of the concentrations measured at the start and at the end of the test.



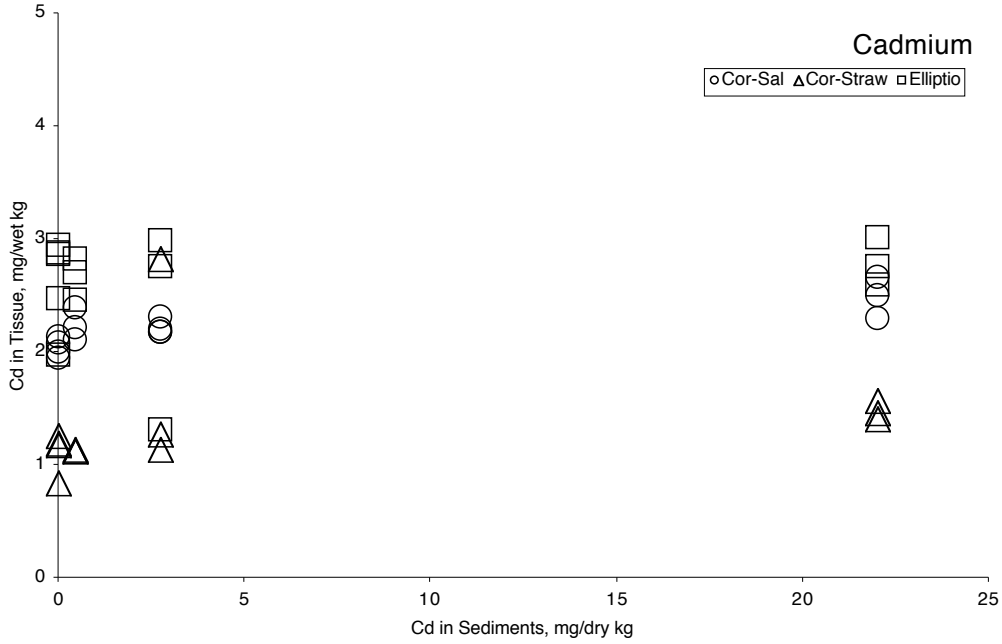
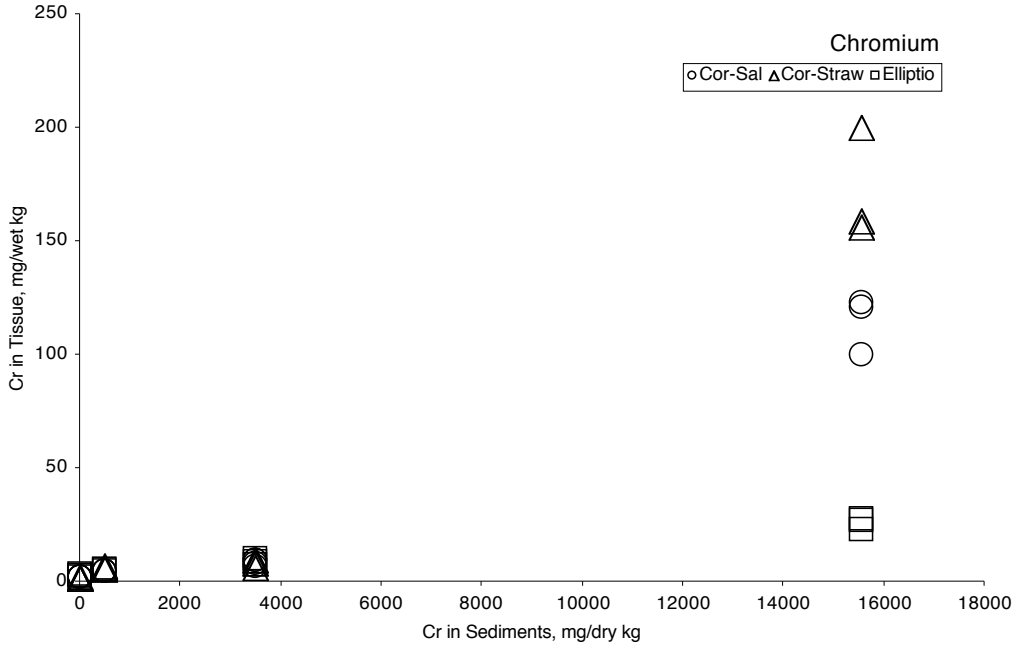


Figure 6 (continued). Comparison among the concentrations of the metals measured in the tissues of the bivalves to the concentrations in the sediments to which they were exposed. The sediment concentrations are the average of the concentrations measured at the start and at the end of the test.



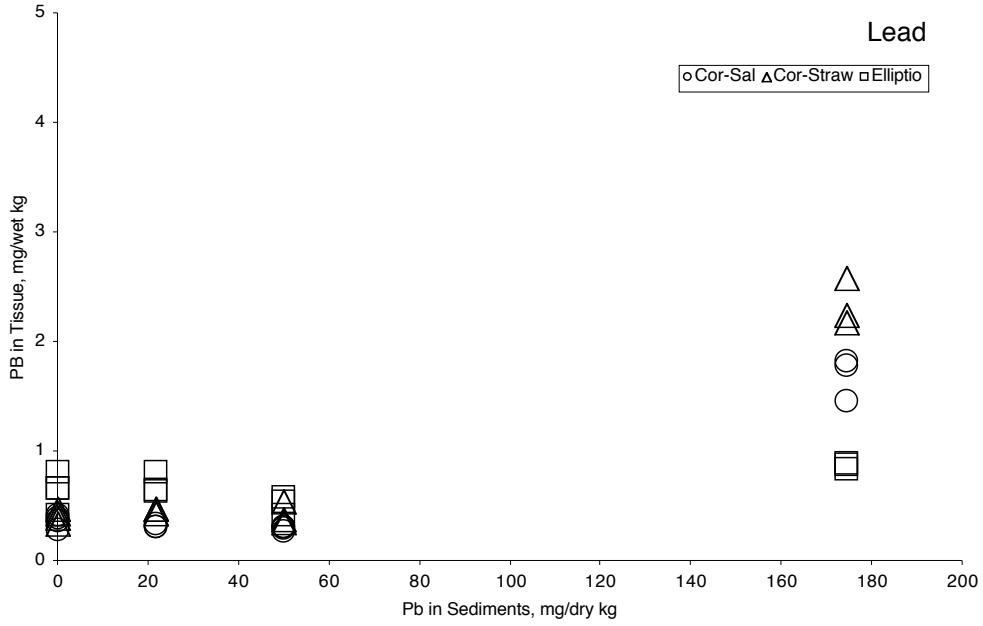


Figure 7. Regression describing the correlation between *Corbicula* and *Elliptio* change in Cr concentration.

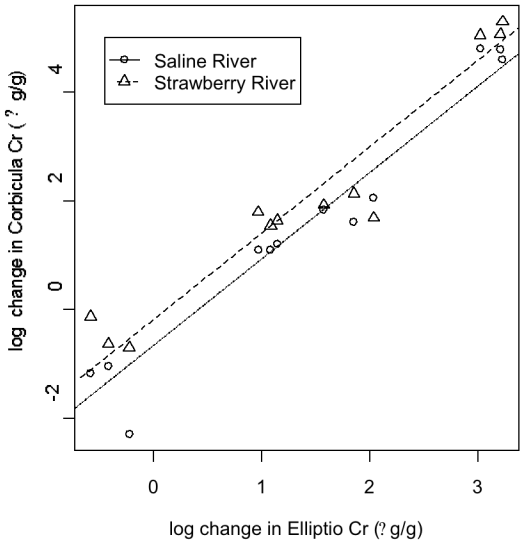


Figure 8. Regression describing the correlation between *Corbicula* and *Elliptio* change in Cr mass.